

Application of the *in-vivo*-haploid induction in hybrid maize breeding

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APPENDIX 1

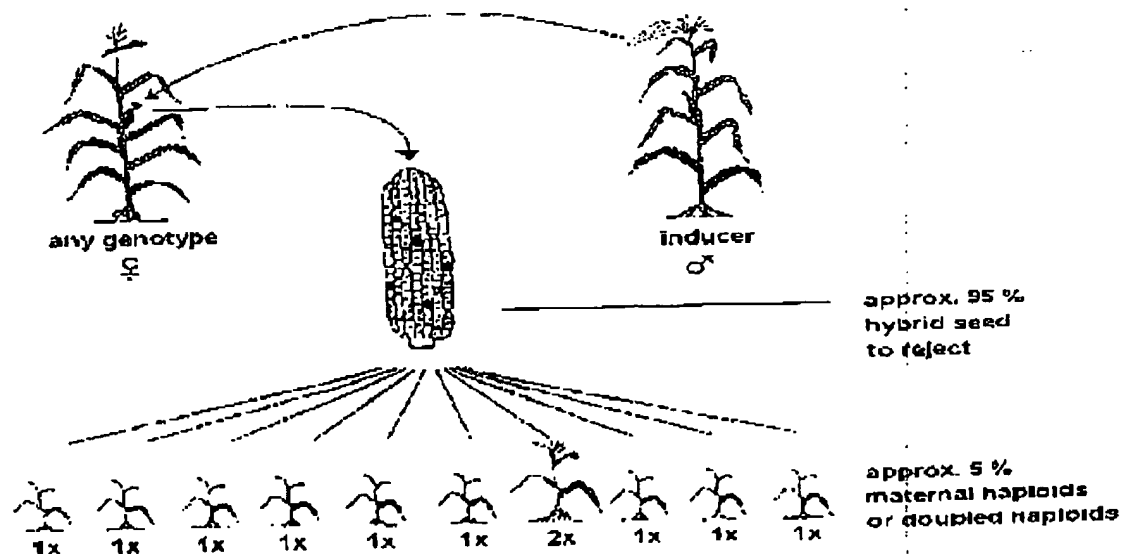
1. Reproductive and genetic investigations on *in-vivo*-haploid induction in maize (*Zea mays* L.)

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DH-Line in generation D₁

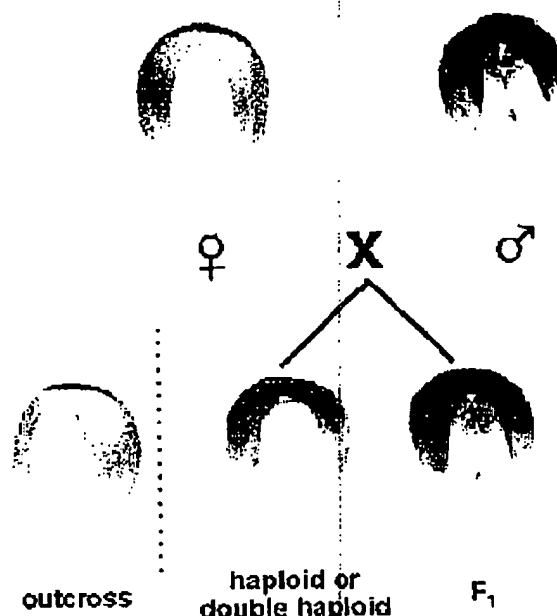
The interest in haploid/double haploid (H/D) techniques has enormously increased in the last years. The introduction of H/DH-techniques in maize breeding programs traces back to the 50s. Shortly after the first reports of the spontaneous occurrence of H/DH-plants in maize, scientists and breeders started to discuss the application of such homozygous plants in breeding programs and their commercial use. By means of the development of inducers and a method for artificial doubling of chromosome set, the H/DH-technique has been developed in the past years until such an extent that it is being used as a matter of routine by maize breeders.



After pollination with an inducer plant, kernels with H-embryo of maternal origin with triploid endosperm arise, together with regularly

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double fertilized kernels. Chromosome elimination and parthenogenesis are considered to be the possible biological mechanisms responsible for the occurrence of H-plants. However, chromosome elimination and parthenogenesis exclude each other per definition. Therefore, we chose the neutral term *in-vivo-haploid* induction for the phenomenon mentioned.



Inductor RWS

The aim of our work was to develop a novel inducer line with an increased induction rate. The inducer line RWS developed, displays both advantage of a high induction rate and combination of two dominant identification markers a red stem, and an embryo and endosperm coloration. Inducer RWS enables the breeder to use *in-vivo-haploid* induction as an effective tool for development of H/DH-plants with almost any genetic background. The method is less effective with donor genotypes, carrying the above mentioned identification markers or anthozya inhibitor-genes themselves.

The spontaneous doubling rate in maize ranges from 1-10 %. Therefore an artificial chromosome doubling method to increase the number of fertile DH-plants is essential. The artificial chromosome doubling method, using colchicine as doubling agent, facilitates an effective development of DH lines.

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Identification of H/DH-plants based on lacking stem-coloration



H/DH-field

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